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Evaluation of Mutagenicity Testing with *Salmonella typhimurium* TA102 in Three Different Laboratories

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Thirty compounds of various chemical classes were investigated for mutagenicity in a collaborative study (three laboratories) using *Salmonella typhimurium* TA102. With five compounds, hydrazine sulfate, phenylhydrazine, hydralazine, glutardialdehyde, and glyoxal, mutagenicity was detected by all laboratories. Formaldehyde was assessed as weakly mutagenic in only one of three laboratories. The remaining 24 agents were uniformly described as nongenotoxic in TA102. In spite of the overall good qualitative agreement in the mutagenicity results between the three laboratories, some quantitive discrepancies occurred in the dose response of the mutagenic compounds. Varying inter- and intralaboratory differences in the spontaneous rate of revertants were obtained. The usefulness of the tester strain TA102 in routine mutagenicity testing is discussed.

Introduction

The development of the new tester strain *Salmonella typhimurium* TA102 prompted consideration of using this bacterial strain on a routine basis in the Ames test (*1,2*). The tester strain has a different specificity from other Salmonella strains routinely used in mutagenicity screening. It detects a variety of oxidative mutagens and seems to be more sensitive to some aldehydes (*3*) and some DNA-damaging compounds, which are negative with other Salmonella strains (*2*).

A mechanism other than genotoxicity can mimic a mutagenic response in TA102, leading to false positive results due to an increase in pAQ1 plasmids in the cell (*4*). A doubling of the spontaneous mutation rate per plate occurred after pretreatment with high levels of tetracycline (*3*). According to the authors, this effect was presumably caused by an increased copy number of the pAQ1 plasmid (*3*). About 30 copies of the mutant gene are available in TA102. According to the literature, the spontaneous frequency of revertants in TA102 is about 240–320 revertants per plate.

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This collaborative study was perfomed in three laboratories. Thirty compounds of various chemical classes were selected to determine the usefulness of the strain TA102 for screening purposes in mutagenicity testing. Ten compounds were used because they had been tested in a battery of short-term tests in another collaborative study of short-term tests for carcinogens published in 1985 (*5*) by WHO (World Health Organization), IPCS (International Programme on Chemical Safety): hexamethylphosphoramide (HMPA), *o*-toluidine, safrol, diethylstilbestrol (DES), benzene, acrylonitrile, diethylhexylphthalate (DEHP), and phenobarbital. These compounds were evaluated as carcinogens being essentially nonmutagenic for Salmonella with a weak response in TA102 only with acrylonitrile (*6*). Two noncarcinogens used in the WHO study, caprolactam and benzoin, where detailed cancer bioassays in rats and mice are available, served as presumptive negative controls. Acryliamide was selected as a compound that is not mutagenic in standard Ames tests but is known for clastogenic *in vivo* (*7,8*).

Several aldehydes and aldehyde precursors were included in the study: formaldehyde, acroleine, glyoxal, glutardialdehyde, anisaldehyde, vinylacetate, and methylvinylketone as a β-unsaturated (acroleine analogue) ketone, which recently has been described as a mutagen in *Salmonella typhimurium* strain TA100 (*9*). HMPA is a powerful nasal carcinogen viewed as a compound with genotoxic potential by releasing formaldehyde within nasal cells.

Hydrazine and hydroxylamine were reported earlier as mutagenic in TA102 (*10*) but not in other tester strains. Therefore, these compounds and the hydrazine derivatives, hydralazine and phenylhydrazine, were included in the study to investigate the usefulness of TA102 to detect the genotoxic potential of these compounds.

Aromatic amines often produce poor results in standard Amestests. Therefore, aniline was selcted and in addition, *m*- and *p*-toluidine due to their structural similarly to *o*-toluidine used in the WHO/IPCD study. Butanol, though a potential precursor of butyric aldehyde, was selected as likely to be negative; 2-aminoanthracene and mitomycin C served as positive controls.

The compounds were judged as mutagenic if a reproducible, dose dependent increase in the number of revertants was observed. This increase had to be about twice the spontaneous rate or at least 200 colonies over background to meet the criterion for mutagenicity. In the few cases where the increase was between 100 and 200 μg/plate above background, this effect was judged as weak effect (w+). Statistical analysis of the data was not made because no appropriate statistics are suggested in the original papers (*5*).

Material and Methods

All chemicals used for testing were of the highest purity available. The chemical sources and CAS numbers are as listed in Table 1.

The phenotypically characterized tester strain TA102 was obtained from B.N. Ames (University of California, Berkeley, CA). The original strain was sent directly to the three collaborating laboratories. All experiments were done from the original stock culture (without further subculture). No further safeguard checks of phenotype consistencies were made.

The compounds were tested for mutagenicity using the procedure of the Salmonella/mammalian-microsome mutagenicity test as described (*1*) and as specified for TA102 (*3*). Bacteria were grown in nutrient broth, and aliquots were frozen at −80°C. All experiments were performed from the same frozen batch except where stated otherwise. For metabolic activation, a 10% S9 mix from livers of Aroclor-1254-induced Sprague-Dawley rats was used.

All compounds were tested in at least two independent experiments using five doses and three plates per dose. The solvents used for diluting the test compounds were water, ethanol, or dimethyl sulfoxide. All compounds were originally tested up to 5000 μg per plate whenever possible. Limitations were given by cytotoxicity or precipitation.

Table 1. Chemicals tested.

Chemical	CAS No.	Supplier
Acrolein	107-02-8	Merck
Acrylamide	79-06-1	Merck
Acrylonitrile	107-13-1	Merck
Aniline	62-53-3	Merck
Anisaldehyde	123-11-5	Merck
Benzene	71-43-3	Merck
Benzoin	119-53-9	Merck
Butylalcohol	71-36-3	Aldrich
Caprolactam	105-60-2	Merck
Diethylhexylphthalate	117-81-7	Merck
Diethylstilbestrol	56-53-1	Sigma
DMPU	7226-23-5	Merck
Formaldehyde	50-00-0	Merck
Glutardialdehyde	11 1-30-8	Merck
Glyoxal	107-22-2	Merck
HMPA	680-31-9	Sigma
Hydralazine-HCl	304-20-1	Aldrich
Hydrazinsulfate	10034-93-2	Aldrich
Hydroxylamin	5470-1 1-1	Sigma
Isoniacid	54-85-3	Merck
Limonen	5989-27-5	Fluka
Methylethylketon	78-93-3	Merck
Methylvinylketon	78-94-4	Merck
Phenobarbital	57-30-7	Merck
Phenylhydrazine	100-63-0	Merck
Safrol	94-59-7	Merck
m-Toluidine	108-44-1	Merck
o-Toluidine	95-53-4	Merck
p-Toluidine	106-49-0	Merck
Vinylacetate	108-05-4	Merck

Results and Discussion

The results obtained independently in three laboratories are summarized in Table 2. Six of 30 chemicals tested

Table 2, Mutagenicity results of 30 compounds with TA102 in 3 independent laboratories.[a]

	Results[b]		
Compound	Lab 1	Lab 2	Lab 3
Acrolein	− (W)	− (W)	− (E)
Acrylamide	− (W)	− (W)	− (W)
Acrylnitrile	− (W)	− (E)	− (E)
Anilin	− (E)	− (E)	− (E)
Anisaldehyde	− (D)	− (E)	− (E)
Benzene	− (D)	− (W)	− (D)
Benzoin	− (D)	− (D)	− (D)
Butanol	− (W)	− (E)	− (W)
Caprolactam	− (W)	− (W)	− (W)
Diethylhexylphthalate	− (D)	− (E)	− (D)
Diethylstilbestrol	− (D)	− (E)	− (D)
DMPU	− (W)	− (W)	− (D)
Formaldehyde	W+(W)	− (W)	− (W)
Glutardialdehyde	+(W)	+(W)	W+(E)
Glyoxal	+(W)	+(W)	+(W)
HMPA	− (W)	− (W)	− (D)
Hydralazine-HCl	+(D)	+(W)	+(W)
Hydrazin-sulfate	+(D/W)	+(W)	+(W)
Hydroxylamine	− (W)	− (W)	− (W)
Isoniacid	− (W)	− (W)	− (W)
Limonen	−(D/E)	− (E)	− (D)
Methylethylketon	− (W)	− (W)	− (E)
Methylvinylketon	− (W)	− (E)	− (E)
Phenobarbital	− (W)	− (W)	− (W)
Phenylhydrazine	+(W)	+(W)	+(D)
Safrol	− (E)	− (E)	− (D)
m-Toluidine	− (D)	− (E)	− (E)
o-Toluidine	−(D/E)	− (E)	− (E)
p-Toluidine	−(D/E)	− (E)	− (W)
Vinylacetate	− (E)	− (E)	− (E)

[a]Solvent used in parentheses: (W) water, (E) ethanol, (D) dimethylsulfoxide.
[b] −, Not mutagenic; +, mutagenic (> 2-fold of <200 colonies/plate over control); W+, weak effect (100–200 colonies/plate over control).

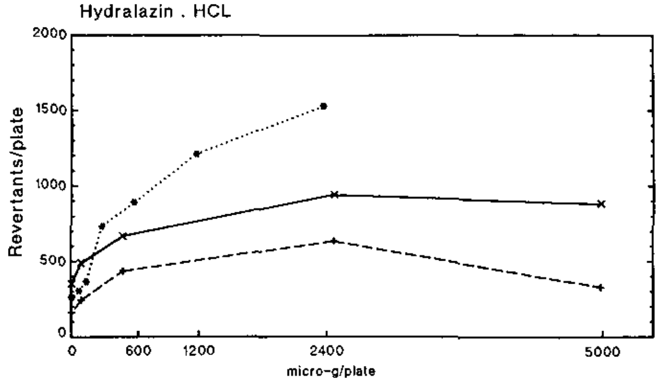

FIGURE 1. Mutagenicity of hydralazin-HCl in *Salmonella typhimurium* strain TA102 with S9 mix in Lab 1 (· · ·), Lab 2 (- - -), Lab 3 (—).

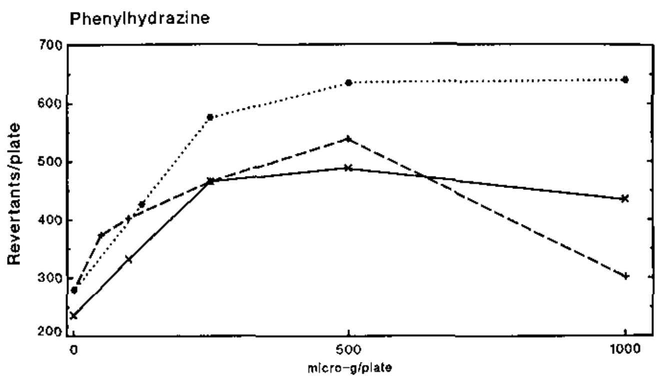

FIGURE 2. Mutagenicity of phenylhydrazine in *Salmonella typhimurium* strain TA109 with S9 mix in Lab 1 (· · ·), Lab 2 (- - -), Lab 3 (—).

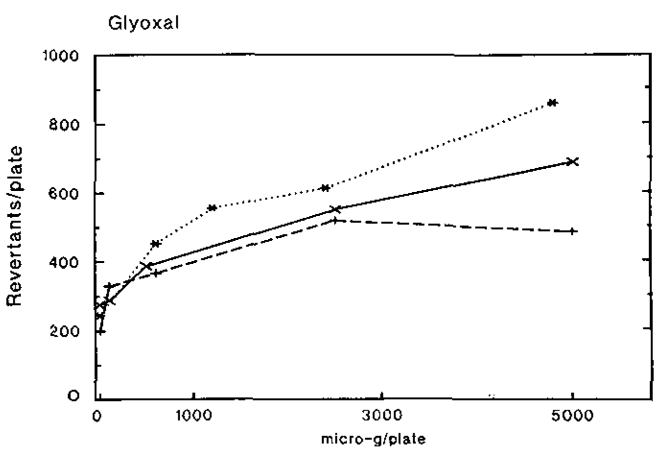

FIGURE 3. Mutagenicity of glyoxal in *Salmonella typhimurium* strain in TA102 with S9 mix in Lab 1 (· · ·), Lab 2 (- - -), Lab 3 (—).

showed mutagenic activity. Hydralazine, phenylhydrazine, and glyoxal were described uniformly as mutagenic in all three laboratories. The dose-response curves for these three compounds are given in Figures 1–3.

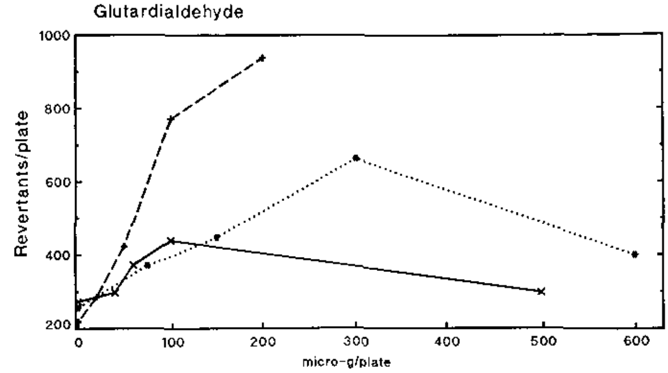

FIGURE 4. Mutagenicity of glutardialdehyde in *Salmonella typhimurium* strain TA102 with S9 mix in Lab 1 (· · ·), Lab 2 (- - -), Lab 3 (—).

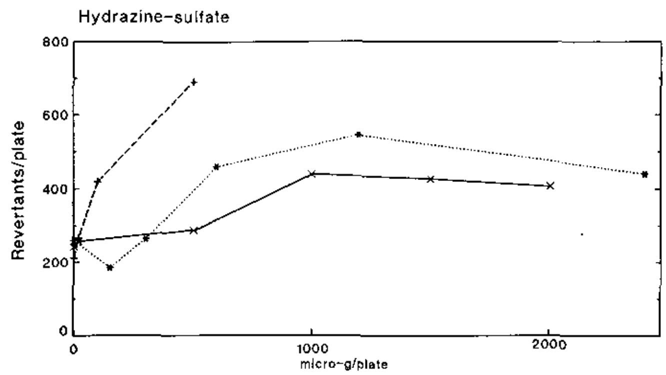

Figure 5. Mutagenicity of hydrazine sulfate and in *Salmonella typhimurium* strain TA102 with S9 mix in Lab 1 (· · ·), Lab 2 (- - -), Lab 3 (—).

In all cases mutagenicity of the compounds was equal or more pronounced in the presence of liver S9 mix. The maximum response with hydralazine-HCl revealed a significant difference in the number of revertants between the three laboratories at the dose level of 2400 μg/plate (laboratory 1) and 2500 μg/plate (laboratories 2 and 3; Fig. 1). The highest increase in revertants was reported in all three cases at this dose. However, in laboratory 1 the induced mutation frequency was more than twice as high as in laboratory 2 (2.7-fold) and laboratory 3 (2.1-fold). Higher doses (>2500 μg/plate) were not relevant for testing due to cytotoxicity.

Comparison of the dose-response curve with phenylhydrazine revealed some quantitative differences in terms of maximum revertants per plate (Fig. 2). The induced mutation frequencies, however, were not always consistent in quantitative terms. In particular, at the top dose levels (1000 μg/plate), quantitative differences were obvious. The quantitative differences in the absolute numbers of revertants were minimized when referred to the corresponding control values. In comparison to published data (*3*), the mutation rate for all the three laboratories was smaller in spite of similar control values.

The coincidence of data with glyoxal was satisfactory up to 2500 μg/plate for all laboratories (Fig. 3). Differences occurred mainly at 5000 μg/plate, where only laboratories 1 and 3 could detect a further increase of mutagenicity. As in the case of phenylhydrazine, the results of these laboratories showed a lower mutagenicity response (*3*).

Glutardialdehyde and hydrazine sulfate were judged as mutagenic in laboratory 1 and 2, whereas only a weak but reproducible increase in the number of revertants was detected in laboratory 3 (Figs. 4, 5). With glutardialdehyde and hydrazinesulfate, laboratory 1 reported the highest effect on the induced numbers of mutation and the mutation frequency. The dose-effect increase was much steeper in the experiments of laboratory 1 than those of laboratories 2 and 3.

Formaldehyde, which has been described as mutagenic (*2,3*), was evaluated as a weak mutagenic compound with and without metabolic activation in one of the participating laboratories only. However, even laboratory 1 had difficulties obtaining a mutagenicity response in the first experiments. The TA102 strain in this laboratory initially had also given negative responses when several different batches of formaldehyde were investigated. Only after testing the compound with a new sample of TA102 (kindly provided by B. Ames) was mutagenicity demonstrated.

In total, the results of our study are in line with recent findings (*11*). We were unable to achieve the high induced revertant counts with several compounds (*3*). In particular, with formaldehyde the maximum-fold increase in TA100 was reported to be only 50% over the control value.

Analysis of the negative controls of all experiments demonstrates relevant variation (Table 2). The spontaneous rate was generally lower in laboratories 1 and 2 than in laboratory 3. Mean control values of all experiments were also lower in laboratories 1 and 2 than in laboratory 3. Comparison of the induced mutations generally reveals the smaller effects in laboratory 3. This may partly be correlated to higher negative control values in this laboratory.

Summary

In summary, there was general agreement in terms of qualitative test results; some quantitative differences were obvious among the results of the three laboratories. The differences in the spontaneous rate of revertants my reflect varying plasmid copies in the bacterial cells.

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